Production of IL-8 and monocyte chemotactic peptide-1 by peripheral blood monocytes. Disparate responses to phytohemagglutinin and lipopolysaccharide.

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The temporal recruitment of leukocytes to a site of inflammation is dependent on a complex interplay of a number of soluble mediators. Recently, two families of chemotactic cytokines have been discovered. The -C-X-C-family, which includes IL-8, appears to recruit neutrophils and lymphocytes. In contrast, the -C-C-family, which includes monocyte chemotactic peptide-1 (MCP-1), appears to recruit predominantly monocytes. Monocytes, after their arrival at a site of inflammation, could further amplify the immune response by secreting IL-8 and MCP-1. We sought to define conditions under which human peripheral blood monocytes produce IL-8 and MCP-1. Using serum-free media, we found that PHA-stimulated monocytes expressed MCP-1 and IL-8 protein and mRNA in a dose-dependent manner. However, the onset of mRNA expression for MCP-1 occurred at least 3 h later than did the onset of IL-8 mRNA expression. IL-8 and MCP-1 gene expression by monocytes appeared to require de novo protein synthesis, in that cycloheximide blocked the expression of mRNA for both IL-8 and MCP-1 in PHA-stimulated cells. However, treatment of monocytes with cycloheximide resulted in the superinduction of IL-8 compared with control monocytes. Monocytes costimulated with PHA and LPS demonstrated enhanced amounts of IL-8 mRNA and protein, but sharply decreased amounts of MCP-1 mRNA and protein. The addition of serum to culture media increased both the constitutive and PHA-induced production of monocyte-derived MCP-1 and IL-8, but had no effect on the inhibition of PHA-stimulated MCP-1 production by LPS. These findings suggest that distinct pathways of activation exist for the production of monocyte-derived IL-8 and MCP-1. The differential expression of these different but related polypeptides may offer a means of control of the type of immune cells that are recruited to a site of inflammation.

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a. Inventions I and III are independent and distinct, each from the other, because they are products which possess characteristic differences in structure and function, and each has an independent utility, that is distinct for each invention which cannot be exchanged.

b. Inventions II and IV are independent and distinct, each from the other, because the methods are practiced with materially different starting materials, have materially different process steps, and are for materially different purposes.

c. Invention I is unrelated to inventions II and IV. Inventions are unrelated if it can be shown that they are not disclosed as capable of use together and they have different modes of operation, different functions, or different effects (MPEP § 806.04, MPEP § 808.01). In the instant case the different inventions are not disclosed as capable of use together.

d. Inventions II and III are independent and distinct, each from the other, because the structure of the compound is independent of the means of identifying it, especially as the functional assay of group II would reasonably be expected to identify compounds having distinct structures and functions.

e. Inventions III and IV are unrelated. Inventions are unrelated if it can be shown that they are not disclosed as capable of use together and they have different modes of operation, different functions, or different effects (MPEP § 806.04, MPEP § 808.01). In the instant case the different inventions are not disclosed as capable of use together.

f. Because these inventions are distinct for the reasons given above and have acquired a separate status in the art because of their recognized divergent subject matter and separate classification, restriction for examination purposes as indicated is proper.